AMENDMENTS TO THE SPECIFICATION:

Please add the following new paragraph on page 1 after the title of the application and before the first line of the application, which begins on page 1:

This application is a national stage filing under 35 U.S.C. § 371 of International Application No. PCT/FR00/00430 filed on February 21, 2000. This application also claims benefit for foreign priority under 35 U.S.C. § 119 and/or 35 U.S.C. § 365 to Application No. 99/02167 filed in France on February 22, 1999.

Please replace the paragraph beginning on page 15, line 1 and ending on page 15, line 21, with the following amended paragraph:

According to the gel filtration chromatography step, the sample is treated on a solid support comprising beads with a diameter of between 3 and 160 μm, advantageously between 5 and 105 μm, and preferably between 10 and 80 μm. Preferably, this support has a porosity close to the size of the virus so that the latter does not penetrate into the beads. On the other hand, the molecules which are smaller in size penetrate into the beads and the migration thereof is slowed. Various types of support may be used, such as matrices based on agarose (Sepharose™), on dextran (Sephadex™ gel), on acrylamide (Sephacryl™ and Trisacryl gels), on silica (TSK and SW gels), on ethylene glycol/methacrylate copolymers (~~Biosec~~ BioSEC, Toyopearl® HW, TSK and PW gels) and on mixtures, in particular mixtures of agarose and dextran (Superdex™ gel). The supports mentioned are preferably used without the functionalization group. The gel filtration

chromatography supports which are particularly suitable for carrying out the preparation method according to the invention are as follows:

Please replace the paragraph beginning on page 15, line 23 and ending on page 15, line 29, with the following amended paragraph:

- allyl dextran/methylene bisacrylamide matrices (Sephacryl™ S300 HR with a bead diameter of between 25 and 75 μm, Sephacryl™ S400 HR with a bead diameter of between 25 and 75 μm, Sephacryl™ S500 HR with a bead diameter of between 25 and 75 μm and Sephacryl™ S1000 SF with a bead diameter of between 40 and 105 μm;l Pharmacia),

Please replace the paragraph beginning on page 15, line 31 and ending on page 15, line 33, with the following amended paragraph:

- ethylene glycol/methacrylate matrices (Toyopearl® HW 55, Toyopearl® HW 65 and Toyopearl® HW 75, with a bead diameter ranging from 20 to 60 μm; Tosohaas),

Please replace the paragraph beginning on page 16, line 1 and ending on page 16, line 2, with the following amended paragraph:

- agarose matrix (Macro-Prep SE with a bead diameter of between 20 and 80 μm; ~~Biorad~~ Bio-Rad).

Please replace the paragraph beginning on page 16, line 4, and ending on page 16, line 23, with the following amended paragraph:

By way of indication, it will be noted that a support of the Toyopearl® HW65F or S (porosity 1000 Å) or Sephacryl™ S400 HR type is preferred. Such a column is equilibrated in a buffer exhibiting saline conditions and a pH limiting the hydrophobic interactions between the support and the viral particles. Advantageously, use will be made of a 25 mM Tris-HCl buffer containing 2 mM $MgCl_2$, 2% sucrose, at pH 8.5, or a 10 mM Tris-HCl buffer containing 10 mM sodium aspartate, 54 mg/1 of Tween® 80 and 2% sucrose, at pH 8.5. The viral particles of interest are eluted without being retained and leave the column before the contaminants of lower molecular weight or smaller size. According to an optional embodiment, the viral fractions obtained after the purification step may be pulled and optionally concentrated according to conventional techniques. Mention may be made of tangential ultrafiltration and diafiltration. The BioMax™ PES (Millipore reference PXB300C50) and PLCMK (Millipore reference PXC300C52) cassettes are most particularly suitable.

Please replace the paragraph beginning on page 17, line 35 and ending on page 18, line 26, with the following amended paragraph:

The protocol according to the invention may also comprise a clarification step, the aim of which is to remove insoluble matter (cellular debris, flocculates of macromolecules, etc.) possibly produced during the cell rupture or lysis step. It may be carried out using any

conventional technique of filtration (depth filtration, tangential microfiltration, etc.) or centrifugation (continuous centrifugation, etc.) Many filters may be used on the condition that they have a porosity which enables the viral particles to pass through and the insoluble matter to be retained. It is indicted that adenoviral particles are approximately 0.07 to 0.1 μm in size, which necessitates the use of filters with a high porosity. Furthermore, the filters may be made of synthetic material (nylon), organic material (cellulose) or nonorganic material (zirconium). According to an advantageous embodiment, successive filtrations are carried out over filters with decreasing porosity, for example initially over a filter with a porosity of 8 μm (Sartorius® 5591301P5-00) then over a filter with a porosity of 5 μm (Sartorius 5591342P5-00), then over a filter with a porosity of 3-0.8 μm (Sartorious, Sartoclean® capsule 5621304E9-00-A) and then, optionally, over a filter with a porosity of between 0.8 and 0.65 μm (Sartorious, Sartoclean® CA capsule 5621305G9-00-A). According to another variant, the filtration may be carried out by tangential microfiltration over flat membranes or hollow fibers with a porosity greater than the size of the adenovirus. In this regard, Durapore® (Millipore) and Omega™ (Pall) membranes may be used.

Please replace the paragraph beginning on page 19, line 15 and ending on page 19, line 29, with the following amended paragraph:

The protocol for producing viral particles according to the invention may also comprise a sterilizing filtration step, said sterilizing filtration step preferably being carried out after

step (c) or (ii) of said preparation method. Use would advantageously be made of 0.22 μm filters. Mention may be made, for example, of filtration units of the Minisart® (Sartorius, reference SM16534), Sartolab® P20 (Sartorius, reference 18053D), Millex® GF (Millipore, reference SLGS025BS), Millex® GV (Millipore, reference SLGV025BS), Millex® GP (Millipore, reference SLGPR25LS) or alternatively Spirale Cap (Super CQS 92 HS or HP version; Gelman Sciences), ~~Criticap~~ CritiCap® 50 (12995, Gelman Sciences) or Millipak® (Millipore, ~~ref.~~ reference MPGL04SK2 or MPGL02SH2) type.

Please replace the paragraph beginning on page 21, line 6 and ending on page 21, line 31, with the following amended paragraph:

The recombinant adenoviruses used in the examples which follow were constructed using the homologous recombination technique described in Chartier et al. (1996, J. Virol. 70, 4805-4810). The constructs used were prepared according to the general techniques of genetic engineering and of molecular cloning, detailed in Maniatis et al., (1989, Laboratory Manual, Cold Spring Harbor, Laboratory Press, Cold Spring Harbor, NY or a more recent edition) or according to the manufacturer's recommendations when a commercial kit is used. The cloning steps use the E. coli strain 5K (hsdR, mcrA), DH5a [(recA1, endA1, hodR17 (r-m-), supE44, thi-1, gryA (nair)] or NM522 (supE, thi, D(lac-proAB), Dhsd5, (r-m-), (F' proAB, $lacI^q$, ZDM15) and those of homologous recombination use the E. coli strain BJ 5183 (Hanahan, 1983, J. Mol. Biol. 166, 557-580). As regards repairing the restriction sites, the technique used consists in filling the overhanging 5' ends

using the large fragment of E. coli DNA polymerase I (Klenow, Boehringer Mannheim). The DNA fragments are purified using the ~~GeneCleanII®~~ GeneCleanII® DNA purification kit (Bio101Inc.). Moreover, the fragments of adenoviral genome used in the various constructs are precisely indicated according to their position in the nucleotide sequence of the Ad5 genome, as disclosed in the Genebank data bank under the reference M73260.

Please replace the paragraph beginning on page 23, line 33 and ending on page 24, line 9, with the following amended paragraph:

The intracellular viral particles are released after rupturing of the cells subjected, from 7 to 10 min, to the mechanical action of a Silverson homogenizer (L4R-Silverson) set at a rotation rate of 4200 rpm. At this stage, the preparation is very viscous due to the release of the genomic DNA following cell rupture. A volume of a buffer which allows optimum action of benzonase and consists of 100 mM Tris, 4 mM $MgCl_2$ and 4% sucrose, pH 8.5, to which has been added the solubilization agent Tween® 80 (Merck reference 8-22187-1000) at a concentration of 2%, is added to the viral preparation. The mixture is stirred at room temperature before adding benzonase in a proportion of 50 U/m (Merck reference 101697) and the reaction is allowed to continue for 1 to 2 h at room temperature and with stirring.

Please replace the paragraph beginning on page 24, line 27 and ending on page 25, line 6, with the following amended paragraph:

Initially, any one of the crude viral preparations obtained in Examples 1 and 2 is subjected to a step for inactivating enveloped viruses. This inactivation step is carried out through the action of TNBP/Tween® 80(tributylphosphate ref.: 24 0494 Aldrich) at a final concentration of 0.3% and 1%, respectively. To do this, the crude viral preparation obtained in Example 1 or 2 is diluted volume-for-volume in a 50 mM Tris buffer solution containing 2 mM $MgCl_2$, 2% sucrose, 450 mM NaCl and 0.6% TNBP (Aldrich 24-049-40), pH 8.5 It is also possible to add to the viral preparation 1/10 of a volume of a more concentrated 50 mM Tris buffer solution containing 1 mM $MgCl_2$, 2% sucrose, 2 M NaCl and 3% TNBP (Aldrich 24-049-40), pH 8.5. It should be noted that the saline conditions used (400 mM NaCl final) correspond to the equilibration conditions of the chromatography. The action of the TNBP/Tween® 80 continues with stirring (500 rpm) for 3 hours at room temperature or for 4 hours at 4°C.

Please replace the paragraph beginning on page 27, line 16 and ending on page 27, line 26, with the following amended paragraph:

The E1-complementation cells are cultured in a bioreactor in ~~Excell~~ ExCell 525 medium (JRH Biosciences) until a concentration of 1 x 10^6 cells/ml is obtained and are then infected with an aliquot of an AdTG13383 prestock, at an MOI of approximately 10. The infected cells and the culture supernatant (volume of approximately 20 l) are harvested 72 h post-

infection. The intracellular viral particles are released after rupturing of the cells subjected, for 7 to 10 min, to the mechanical action of a Silverson homogenizer (275 UHLS) set at a rotation rate of 50 Hz (rate of 8.1).

Please replace the paragraph beginning on page 27, line 28 and ending on page 27, line 37, with the following amended paragraph:

The crude viral preparation thus obtained is subjected to a clarification step in order to remove the insoluble matter (cellular debris, flocculates of macromolecules, etc.). Successive filtrations are carried out over filters of decreasing porosity, first of all over a filter with a porosity of 8 μm (Sartopure® 300PP2 5592501) then over a filter with a porosity of 5 μm (Sartopure® 300 PP3 5592542) and, finally over a filter with a porosity of between 3 and 0.8 μm (Sartorius, Sartoclean CA capsule 5621304E9-00-A).

Please replace the paragraph beginning on page 28, line 1 and ending on page 28, line 14, with the following amended paragraph:

The clarified viral preparation is subjected to a step for degrading the DNA (Benzonase action) and, concomitantly, to a step for inactivating enveloped viruses (action of the 0.3% TNBP/1% Tween® 80 mixture). To do this, one volume of a 100 mM Tris buffer containing 4 mM $MgCl_2$ and 4% sucrose, pH 8.5, comprising Tween® 80 (Merck reference 8-22187-1000) at a concentration of 2%, is added to the clarified viral preparation. The mixture is stirred at room temperature before adding benzonase in a proportion of 10 U/ml

(Merck reference 101697) and TNBP (Aldrich 24-049-40) at a final concentration of 0.3%. The reaction is allowed to continue for 2 h at room temperature with stirring (500 rpm).

Please replace the paragraph beginning on page 29, line 16 and ending on page 29, line 21, with the following amended paragraph:

The fractions obtained after the fluidized-bed chromatography and containing the adenoviral particles are concentrated by diafiltration on Labscale™ (Millipore) using the BioMax™ PES cassettes (Millipore reference PXB01MC50) or cellulose membranes with a cutoff threshold of 300 kDa and 1000 kDa.

Please replace the paragraph beginning on page 29, line 23 and ending on page 30, line 17, with the following amended paragraph:

The concentrated viral preparation is then subjected to gel filtration chromatography. To do this, the viral preparation is loaded onto a column containing a resin of the Toyopearl® HW65F type (ref. Tosohaas 07 465) pre-equilibrated using a 10 mM Tris buffer containing 54 mg/1 Tween® 80, 2% sucrose and 10 mM sodium aspartate, pH 8.5. The buffer is introduced through the top of the chromatography column and is made to leave through the bottom. A flow rate of 30 cm/h is used to equilibrate and load the column with the concentrated viral preparation. The viral preparation applied on to the column (approximately 20% of the column volume) is then rinsed with the buffer which enables the column to be equilibrated (10 mM Tris, 54 mg/1 Tween® 80, 2% sucrose, 10 mM sodium

aspartate, pH 8.5) in the descending direction. The aim of this operation is to remove the low molecular weight contaminants which have been slowed down by passing through the pores of the gel, unlike the virus which is excluded from the gel beads. The various cell constituents which have been slowed down on the chromatography support are then gradually eluted, still with the same buffer. The eluate is recovered in fractions. Each fraction is analyzed by measuring the adsorbance at 260 and 280 nm. Generally, the first peak detected at 280 and 260 nm contains the adenoviral particles of interest, whereas the protein contaminants detected only at 280 nm are eluted in the second position. The fractions corresponding to the first peak are pulled, optionally concentrated by diafiltration, placed in a buffer with a suitable formulation (for example in saline or isotonic solution) and then filtered over 0.22 μm Sartolab P20 (Sartorius, reference 18053D) and stored until use.